Research Article

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In vitro studies on management of basal stem rot of arecanut caused by *Ganoderma lucidum* (Curtis Ex. Fr.) Karst

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Abstract

Basal stem rot caused by *Ganoderma lucidum* (Curtis Ex. Fr.) Karst is one of the major constraints in arecanut cultivation in Assam. Pure culture of Ganoderma was isolated from fruiting body. The potential biocontrol agents, viz., *Trichoderma harzianum, T. viride* and *Gliocladium virens* were isolated from soil samples of arecanut rhizosphere. Dual culture studies revealed that the three fungal cultures viz., *Trichoderma harzianum* (63.99%), *T. viride* (66.55%) and *Gliocladium virens* (62.12%) have inhibitory effect on the mycelial growth of the pathogen after 96 h of incubation. Standard aqueous extract of twenty plant species were screened and *Azadirachta indica* leaf extract completely inhibited the growth of the pathogen. Extract of *Allium sativum* exerted 64.16 % inhibition over control followed by *Clerodendron infortunatum* (63.82%) and *Centella asiatica* (59.21%) after 96 h of incubation.

Key words: Arecanut, *Ganoderma lucidum*, bio-agents, botanicals

Introduction

The arecanut palm, *Areca catechu* L. is the only cultivated species in the genus *Areca*. It is the source of common masticatory nut popularly known as "Supari" or "betel nut" which is used in many parts of the world. It is an essential requisite for several religious and social ceremonies. Arecanut palm is affected by a number of diseases during different stages of its growth and development. Basal stem rot caused by *Ganoderma lucidum* (Curtis Ex. Fr.) Karst is one of the dreaded diseases of arecanut. It has not only affected the productivity but has also wiped out areca plantations in certain localities. Occurrence of this disease was reported during 1807 in Karnataka (Buchanan, 1807), from Tamil Nadu, Kerala and Assam (Anonymous, 1960), Bengal (Sharples, 1928), Nicobar Islands (Sangal *et al.*, 1961.) Although control measures using fungicides are reported to be effective (Nambiar and Nair, 1973; Kumar and Nambiar, 1990), it becomes very difficult for large scale adoption. Moreover, with the increased awareness on toxic hazards of chemicals to crops, consumers and the environment due to their phytotoxic, residual and pollution effects, exploration of some innovative techniques for the management of disease has become an imperative need. Keeping this in view, the present investigation was undertaken to conduct *in vitro* screening of antagonistic microorganisms and botanicals against the pathogen.

Materials and Methods

Isolation of *G. lucidum* was made from the fruiting body of basal stem rot affected arecanut palm on potato dextrose agar (PDA) media (Fig. 1). The diseased samples were cut into small convenient sized pieces, sterlized in 0.1% $HgCl_2$ for one minute, washed thrice

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Fig. 1. Bracket formation (Sporophore)

in sterile distilled water and plated on PDA medium. The plates were incubated at 28±2°C. Isolation of antagonistic microorganism was done from the soil sample of arecanut rhizosphere, collected from CPCRI, RC, Kahikuchi.

In vitro antagonism of the antagonists against *G. lucidum* was tested by dual culture technique on PDA medium (Dhingra and Sinclair, 1985). A petriplate inoculated with *G. lucidum* alone served as control. Each experiment was replicated three times. Observation on mycelial growth of the pathogen was recorded up to 96 h of incubation. The per cent inhibition over control was calculated.

Twenty locally available botanicals were tested for their antifungal property against *G. lucidum* by poisoned food technique (Bhaskaran *et al.,* 1988) under *in vitro* condition. Fresh leaves of test plants were taken for preparing crude extracts. The leaves were thoroughly washed with water and a fine slurry was prepared by taking 100 g leaves with 100 ml of distilled water. The resultant slurry was filtered through muslin cloth and then through Whatman No. 1 filter paper and the extracts were used as stock solution. From the stock solution, 10 ml of sterilized extract was added with 90 ml of medium aseptically to make 10% concentration. The plant extracts were also assessed for their inhibitory effect, if any, on the antagonistic fungi under *in vitro* condition by poisoned food technique.

Results and Discussion

Effect of bio-agents on mycelial growth of *G. lucidum*

Three antagonistic fungi, *viz. Trichoderma harzianum* (AF1), *T. viride* (AF2) and *Gliocladium virens* (AF3) were isolated from soil samples of arecanut rhizospere The isolates were confirmed according to th identification key (Rifai, 1969) based on the branchin of conidiophores, shape of phialides, emergence o phialospores and shape of phialospores. The three funga cultures isolated from soil were found to have inhibitor effect on the mycelial growth of the pathogen (Table and data showed that degree of inhibition was maximu with *T. viride* (66.55%), followed by *T. harzianu* (63.99%) and *G. virens* (62.12%) after 96 h of incubatio There is significant difference among all the antagonist

Table 1. Effect of different antagonists on mycelial growth of *Ganoder lucidum*

Sl. No.	Antagonistic organisms	Per cent inhibition over control		
		48 h	72 h	96 h
1.	*Trichoderma harzianum*	27.09 (31.36)	42.27(40.47)	63.99(53.1
2.	*T. viride*	35.96(36.85)	44.69(41.95)	66.55(54.6
3.	*Gliocladium virens*	32.51(34.76)	40.49(39.52)	62.12(52.0
SEd		0.12	0.15	0.12
CD(0.05)		0.28	0.37	0.28

The data are the mean of 3 replications.

Data within parentheses are the angular transformed values

Effect of botanicals on mycelial growth of *G. lucidu*

Influence of botanicals on mycelial growth of *lucidum* is presented in Table 2. Results revealed th among the twenty plant extracts tested, *Azadirach indica* extracts completely inhibited the growth of t

Table 2. Effect of plant extracts on mycelial growth of *Ganoderma lucidum*

Sl. No.	Botanicals	Per cent inhibition over control			Mc
		48 h	72 h	96 h	
1.	*Allium sativum*	68.47(55.84)	74.07(59.39)	64.16(53.22)	68.
2.	*Ageratum houstonianum*	36.94(37.43)	29.13(32.66)	29.69(33.02)	31.
3.	*Azadirachta indica*	100.00(90.00)	100.00(90.00)	100.00(90.00)	10
4.	*Bougainvillea spectabilis*	39.90(39.17)	44.69(41.95)	46.76(46.76)	43
5.	*Bryophylium pinnatum*	44.83(42.03)	45.68(42.52)	48.46(44.12)	46.
6.	*Carthamus oxycantha*	49.26(44.57)	57.53(49.33)	56.99(49.02)	54.
7.	*Centella asiatica*	46.30(42.88)	64.20(53.25)	59.21(50.31)	56.
8.	*Clerodendron infortunatum*	45.81(42.60)	65.43(53.99)	63.82(53.02)	58.
9.	*Datura stramonium*	44.83(42.03)	55.31(48.05)	57.68(49.4)	52.
10.	*Eupatorium odoratum*	36.94(37.43)	36.05(36.90)	32.59(34.81)	35.
11.	*Leucas aspera*	49.26(44.57)	55.06(47.90)	51.11(45.63)	51.
12.	*Murraya koenigii*	44.83(42.03)	52.54(46.45)	52.05(46.17)	49.
13.	*Musa* sp.	35.47(36.55)	47.65(43.65)	44.37(41.77)	42.
14.	*Ocimum sanctum*	8.86(17.32)	32.10(34.51)	47.95(43.82)	20.
15.	*Oxalis* sp.	40.89(39.75)	57.04(49.05)	54.61(47.64)	50.
16.	*Polyalthea longifolia*	45.32(42.31)	48.15(43.94)	45.39(42.35)	46.
17.	*Psidium guajava*	38.42(38.30)	55.55(48.19)	49.83(44.90)	47.
18.	*Solanum nigrum*	48.77(44.29)	56.30(48.62)	52.39(46.370	52.
19.	*Tagetes erecta*	42.36(40.60)	52.84(46.63)	55.97(48.43)	50.
20.	*Vitex negundo*	41.87(40.32)	48.15(43.94)	47.61(43.63)	45.
SEd		1.37	0.23	0.19	
CD(0.05)		2.77	0.48	0.38	

The data are the mean of 3 replications.

Data within parentheses are the angular transformed values

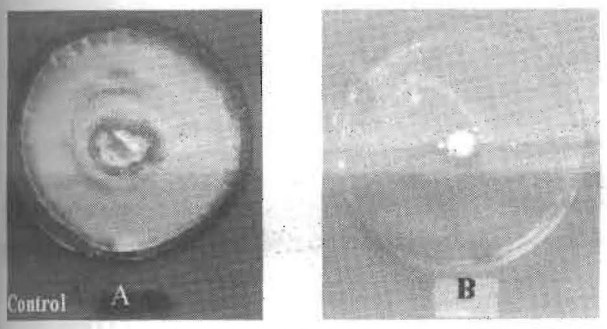

Fig. 2. Mycelial growth of *G. lucidum*: A-control, B-plated with *Azadirachta indica*.

pathogen (Fig. 2). *Allium sativum* showed 64.16% inhibition over control, followed by *Clerodendron infortunatum, Centella asiatica, Datura stramonium* and *Carthamus oxycantha* with 63.82, 59.21, 57.68 and 56.99 per cent inhibition over control after 96 h of incubation respectively. However, there is no significant difference between *A. sativum* and *C. infortunatum* and also between *D. stamonium* and *C. oxycantha*. Iyer *et al.* (2004) reported that among the forty three plant species screened against *G. lucidum* under *in vitro* condition, *Allium sativum, Peperomia pellucida, Clerodendron infortunatum* and *Musa paradisiaca* were found to inhibit the growth of the pathogen. The compatibility study of the antagonistic microorganism with plant extracts showed that all are found to be compatible except the extract of *Allium sativum*. Bhaskaran *et al.* (1988) reported that neem cake extract completely inhibited the growth of *G. lucidum* isolate of coconut. Gunasekaran *et al.* (1986) and Bhaskaran (1990) reported that *T. viride* and *T. harzianum* are potentially antagonistic to *G. lucidum* and can be successfully employed in the bio-control of basal stem rot disease.

Conclusion

From the present study, it can be concluded that the aqueous plant extract of *Azadirachta indica, Clerodendron infortunatum, Centella asiatica, Datura stramonium and Carthamus oxycantha* can be used for managing basal stem rot disease of arecanut in the endemic areas. Moreover, all the plant extracts, except garlic, are compatible with the antagonistic microorganism and therefore, the promising plant extracts may be effectively integrated in disease management strategies.

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